Measuring Transcription Kinetics in *E.coli* Using Synthetic Constructs

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***Short Abstract* — We study the kinetic properties of transcription for various promoters in *E.coli* using synthetic single-copy plasmid constructs. In each construct, the promoter of interest initiates transcription of a region coding for multiple phage coat-protein binding sites. Upon transcription, RNA binding sites are formed, and fluorescent phage coat proteins (MS2, PP7) bind to them. Using single-cell fluorescent microscopy, we can monitor the fluorescence intensity of the bound proteins as a function of time and directly measure the kinetic properties of transcription. Our goal is to explore whether transcriptional apparatus that are characterized by different underlying components yield distinct noise responses.**

I. Purpose

ONE of the intriguing questions in multicellular organism development is how precise differentiation of cells at the early developmental stages can occur when the expression of the proteins that govern differentiation is known to be noisy. Generally, many factors contribute to the protein expression level and to its variance. In this work we focus on transcription and on its contribution to the noise in protein expression.

II. Methodology

WE work with a 2-plasmid system in *E.coli* following the work of Golding *et. al*. [1,2]. The first plasmid codes for a fluorescent RNA-binding phage coat protein (MS2-mCherry, PP7-EGFP) [3]. The second, single-copy plasmid contains a promoter of interest (T7, σ54). The region transcribed under the promoter encodes multiple binding sites for the RNA-binding proteins. Upon transcription, RNA binding sites are formed, and fluorescent coat proteins bind to them. We monitor the number of the fluorescent RNA-binding proteins bound to the transcript as a function of time in many single cells using fluorescence microscopy. Specifically, we measure the transcription rate, and the interval between separate transcription events for different promoters.

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References

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